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Method Development and Subsequent Survey Analysis of Biological Tissues for Platinum, Lead, and Manganese Content

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An emission spectrochemical method is described for the determination of trace quantities of platinum, lead, and manganese in biological tissues. Total energy burns in an argon–oxygen atmosphere are employed. Sample preparation, conditions of analysis, and preparation of standards are discussed. The precision of the method is consistently better than ±15%, and comparative analyses indicate comparable accuracies. Data obtained for experimental rat tissues and for selected autopsy tissues are presented.

Introduction

The rapidly changing environment of man in this industrial-technological age will, in all probability, expose him to many previously exotic elements. The exposure potential for platinum and palladium associated with the anticipated use of catalytic convertors in automobiles is one such case. It is, therefore, worthwhile to establish the tissue levels of these two elements before the potential for environmental alteration has occurred.

A survey of current literature has revealed that there is no published analytical procedure presently available for the determination of platinum and palladium in biological tissues. In fact, data available relative to the residual concentrations of these two elements in biological tissues are scanty, but best estimates place the order of magnitude in the nanogram range (*1,2*). The reason for this situation is twofold—namely, the extremely low concentration of these elements in tissues is below the detection limit of the analytical techniques routinely employed for tissue analysis; and the lack of scientific interest in these elements because of their very limited distribution in the environment and the attendant low environmental exposure probability. Specifically, the concentration of platinum metals in commercial ore is 0.5 ppm (*3*).

An example of an analytical technique of inadequate sensitivity is described by Wolstenholme (*4*), who analyzed dried blood plasma by spark source mass spectrometry. Detection limits of 0.7 ppm for platinum and 0.2 ppm for palladium are reported. A similar situation was found by Gofman et al. (*5*), who used x-ray spectrochemistry for the analysis of blood. Although platinum and palladium were not detected, they reported 99% confidence limits on mean values of 0.00–0.10 ppm for palladium and 0.00–0.46

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ppm for platinum. The simultaneous determination of thirty trace elements (including platinum) in cancerous and noncancerous human tissue samples by neutron activation analysis is reported by Samsahl, Brune, and Webster (*6*). In actuality, only 18 elements were analyzed quantitatively, and no concentration levels were indicated for the other twelve elements. Platinum was in the qualitative group.

With the potential probability of increased environmental exposure to platinum and palladium, it is imperative that analytical methodology be developed quickly which will allow base line concentration levels to be established in biological tissues. From a toxicological standpoint, based on intravenous injections of mammals, palladium has been classified as highly toxic and platinum as moderately toxic (*2*). Durbin (*7*), through the use of radioisotopes of high specific activity demonstrated an uptake of soluble palladium in mammalian liver and kidney. Soluble platinum concentrated in mammalian liver, kidney, and spleen. Insoluble forms of both elements were taken up by lung. It is, therefore, desirable that the technology be available to monitor the concentration of these elements in biological tissues to assure that any potential health hazard will be quickly detected. An analytical method capable of filling this need would, of necessity, possess sufficient sensitivity to cope with and establish the statistical variability inherent in dynamic biological systems.

The objective of this paper is to report the development of an emission spectrochemical procedure for the analysis of platinum, lead and manganese in experimental rat tissues. Although the method does not possess sufficient sensitivity to allow residual baseline platinum concentration levels to be established, it does possess greater sensitivity than previously reported spectrographic procedures (*8–10*). It was developed to serve as a means of evaluating initial exposure experiments on test animals. It will also serve to determine the distribution of manganese, an essential trace metal; lead, a known toxic metal; and platinum, a metal of special interest. The data thus obtained will be invaluable as a guide in the design of future exposure experiments, and ultimately, in determining the direction and approach of future research in the area of fuel additives and their associated environmental and health effects.

Experimental

Selection of Analytical Method

A number of restrictions were imposed at the outset of this program which limited the selection of the analytical approach to be investigated. The most serious restrictions were: (1) a rigid time deadline, i.e., all method development and the analyses of ca. 400 samples (1200 determinations) had to be completed and reported in a period of 6 weeks; (2) the extremely low residual concentration of platinum in biological tissues; (3) the limited amount of tissue sample available for analysis; (4) the multielement (Pt, Mn, and Pb) analysis requirement for each tissue; (5) unknown factors relating to the concentration and distribution of the platinum in the test animals.

Because of the time limitation, the approach selected had to be one where all necessary equipment and supplies were available in-house. The other four limitations dictated the sensitivity requirement of the method. Two possible analytical techniques were considered; flameless atomic absorption and emission spectroscopy.

Flameless Atomic Absorption: Absolute sensitivities of 100–1000 pg for Pt are reported by two major suppliers of flameless equipment (*11,12*). These reported sensitivities were evaluated in terms of the following equivalences: 1000 pg = 1 ng = 1 ppb = 0.001 ppm = reported level of Pt in 10 mg of ash ~ 1.0 g wet tissue.

The usual sample size employed in flameless atomic absorption is 5 μl. To achieve the necessary Pt concentration needed to

give an absolute amount of 100 picograms, the test solution must have a concentration of 20 ng/ml (20 ppb). To obtain 1 ml of solution containing 20 ppb of Pt, all the Pt from 20 g of wet tissue (200 mg of ash) must be concentrated into 1 ml—an impossible situation due to sample size limitations. The use of the maximum sample size for the carbon rod atomizer (20 μl) will reduce the initial sample size requirement to 5 g of wet tissue in 1 ml of solution—an improved but still unacceptable situation.

Theoretically, the technique has a detection limit for Pt equal to the expected concentration level of the samples. To achieve this sensitivity, it will be necessary to concentrate all the Pt contained in a 1-g wet tissue sample into a single 200-μl sample. Use of a preconcentration technique with emphasis on extraneous salt removal is dictated, since ca. 10 mg of inorganic ash will be involved for a sample of 1 g wet tissue. The selection of an appropriate preconcentration scheme could require extensive investigation because of probable complications associated with the insolubility of certain platinum compounds (especially PtO) in acids including aqua regia.

Emission Spectroscopy: Most emission spectrographic techniques used for tissue analysis are developed with one goal in mind—the attainment of data for a maximum number of elements from a minimum amount of sample (*8–10*). It is possible to modify this approach and to develop a custom technique designed to give maximum sensitivity for platinum. The anticipated improvements should place the absolute detection limit somewhere between 0.010 and 0.025 μg/electrode.

In actuality, neither analytical approach will reach the desired residual platinum concentration level without a preconcentration treatment in addition to ashing. However, the time limitation imposed on the project was so severe that an adequate investigation of preconcentration schemes was not feasible. Consequently, the approach selected represents a compromise; but, in view of the limited sample sizes available and the multielement analysis requirement, it best meets the needs of the project.

Table 1. Analytical Variables examined and conditions selected.

Variable	Range of Study	Optimum Conditions
Arc current, A	5–30	12.5
Electrode polarity	—	Sample anode
Slit width, μ	10–50	25
Electrode composition	Graphite, carbon	Carbon
Electrode geometry	Variable	Ultra C-7314 (Ultra Custom 7437-cathode)
Exposure time, sec	5 (Total burn)	Initial, 30 (rack camera without interrupting burn); continue to completion of total burn
Analytical gap, mm	4–7	5
Sample size, mg	10–40	30
Spectroscopic buffer, carrier, or diluent	Graphite, Li_2CO_3–graphite (1:5), In_2O_3–SrF_2 (1:2), graphite–NaBr (1:1)	Graphite
Sample: buffer ratio	1:1–1:4	1:2
Gas composition	Mixtures of O_2 with A and He	80% A, 20% O_2
Gas flow rate, l/min	2–10	10
Emulsion	Kodak SA1 and SA3	Kodak SA1

The approach selected was the development of a custom emission spectrographic method which would yield optimum sensitivity for platinum. It is recognized that the proposed method will not yield positive values for small tissue samples (<1 g) which contain Pt at the anticipated residual concentration level (0.001 ppm). The proposed approach will, however, easily detect concentrations of platinum at levels which can be defined as representing values significantly higher than the normal statistical variation for a dynamic biological system. No problems are anticipated with the analysis of lead and manganese by the proposed method.

Selection of Analysis Conditions

The dc arc emission spectrographic meth-

Table 2. Experimental apparatus and conditions.

Excitation system	
Source unit	Jarrell-Ash custom varisource (Model 40–750–S)
Discharge	12.5 A dc arc (shorted electrodes)
Sample electrode	Ultra C–7314
Counter electrode	Ultra custom 7437
Analytical gap, mm	5
Exposure time	Initial exposure, 30 sec, rack without interrupting burn, total burn
Excitation chamber	Modified Spex enclosed Stallwood jet (*13*)
Gas mixture	10 l./min, 20% O_2–80% A
Electrode-chamber base distance, mm	12
Optical system	
Spectrograph	Jarrell-Ash 3.4 m Ebert (Model 71–000)
Grating, lines/in.	15000
Wavelength coverage, A	2300–4550
Slit width, μ	25
External optics	Two-lens with adjustable diaphragm to mask electrode tips
Detector	35 mm Kodak spectrum analysis No. 1 film
Developing conditions	
Development	Eastman D–19 (full strength), 3 min
Stop bath	5% Acetic acid, 1 min
Fixing	Eastman Rapid Fixer with hardener, 4 min
Densitometer	Jarrell-Ash microphotometer (Model 21–000)
Sample preparation	
Freeze-dryer	Virtis freeze dryer (Model USM–15)
Grinding and blending	Spex mixer/mill (No. 8000–11)

ods employed for tissue analysis by Bedrosian et al. (*8*), Morrison et al. (*9*). Koirtyohann and Feldman (*10*), as well as the in-house method of Stewart Laboratories, Inc. were surveyed to determine which parameters appeared to make the greatest contributions to the enhancement of the sensitivity of the methods. The analytical variables examined and the optimum conditions selected are listed in Table 1. In all instances the criterion for judgement was the line/background ratio of the Pt 3064.79 line.

Volatilization rate studies indicated that the line/background ratio for lead, as well as platinum, could be optimized by employing a split burn technique. After the initial 30 second exposure, the film is racked one position without interrupting the burn. The remainder of the total burn is recorded on this second exposure. Volatilization profiles for the elements under study are shown in Figure 1.

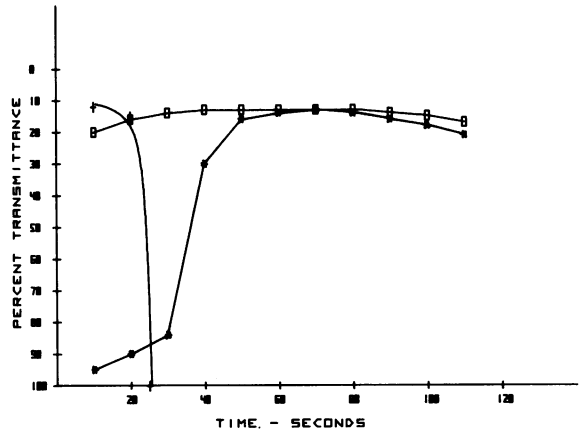

FIGURE 1. Volatilization profiles for (*) platinum, (+) lead, and (□) manganese.

Apparatus

The instrumentation and operating parameters employed are listed in Table 2.

Preparation of Standards

Two matrices were employed in the standardization procedure: an actual soft tissue ash composite and a synthetic soft tissue matrix.

Composite Tissue Ash Matrix. Spex Industries Noble Metals Mix (9.32% Pt) was added to a composite tissue ash. Suitable dilutions were made with the ash composite to provide standards for the method development studies.

Synthetic Matrix. Because of the residual manganese in the composite tissue ash, a synthetic soft tissue ash was prepared. This matrix, identical to that employed by Koirtyohann and Feldman (*10*), is composed of

67% KH_2PO_4, 25% NaCl, 3.6% MgO, 3.0% $CaCO_3$, and 1.4% Fe_2O_3 (all of spectrographic purity). These materials were mixed and heated at 550°C for 16 hr, after which they were thoroughly ground and used as a matrix for the standards.

Four master standards were prepared by adding appropriate amounts of Fisher Certified standard solutions to a slurry of 1 g synthetic matrix in 10 ml of triply distilled water. After thorough mixing, the spiked slurries were transferred quantitatively to silica dishes and evaporated to dryness at 120°C. The dried standards were mechanically blended to insure homogeneity. Working standards were prepared from these four masters with concentrations in the ratio of 0, 1, 2, 5, 7, 10, 20, 50, 70, and 100.

Internal Standard. A number of noble metals, including Au, Ir, Pd, and Ru, as well as Ge, were investigated as possible internal standards. Germanium, as high purity germanium dioxide, was selected as the best compromise for use with the three elements under investigation because of its medium volatility, spectral simplicity, and its rarity in biological tissues. Previous work has verified that germanium possesses desirable characteristics as an internal standard (*14, 15*).

Experimental Animal Tissues

All animal work was directed by Dr. David J. Holbrook, Jr., Department of Biochemistry, School of Medicine, University of North Carolina. Experimental rats received controlled diets and measured dosages of several solutions of platinum, manganese, or lead for varying time periods. Blood was drawn immediately after sacrifice, and the tissues of interest were dissected and weighed. Each tissue specimen was quick-frozen in glass vials with aluminum insert plastic screw caps. Samples remained frozen until they were processed in the analytical laboratory.

Sample Preparation

All tissue samples were lyophilized as soon as they were received. In those cases where sample size permitted, the lyophilized sample was carefully mixed; and a portion of sample equivalent to 0.25 g wet tissue was removed for confirming analysis by an independent technique (atomic absorption). Sample splits used for the confirming analyses were burned by oxygen-flask combustion; and the resulting solutions were analyzed by conventional atomic absorption for manganese, lead, and platinum.

The samples, as received for analysis, ranged in size from 0.17 to 2.60 g. Since the experiments were conducted with paired animals, samples less than 0.80 g wet weight were pooled after freeze-drying, and the composite was ashed for emission analysis as a single sample.

A modified "wet" ashing technique using quartz distilled sulfuric acid was employed. The lyophilized sample is weighed into a previously conditioned silica dish. Enough acid is added to wet the surface of the sample (6 to 10 drops is adequate), and the dishes are placed on hot plates under infrared heat lamps. As soon as the samples have stopped smoking (about 30 min), they are placed in a muffle furnace at 550°C and held at this temperature until ashing is complete. The ash is then weighed and is ready to be prepared for spectrochemical analysis.

Two advantages are experienced when tissue samples are freeze-dried prior to ashing: the time required for ashing is reduced by a factor of three and the disagreeable odors created by conventional drying and ashing procedures are greatly reduced.

The incorporation of the internal standard material, germanium dioxide (Spex Industries High Purity GeO_2), in the graphite matrix mixture is accomplished by successive dilutions of a master mixture containing 5% germanium dioxide. The working matric contains 0.002% germanium in high purity conducting graphite (0.4 μg Ge per electrode).

Electrode charges are prepared by care-

fully mixing 20 mg of working matrix with the total ash for the sample (for ash weights up to 15 mg). For ash residues 15 mg and greater, a 1:2 ash to matrix mixture is prepared, and 30 mg of the resulting mixture is packed into the sample electrode. Less than 10% of the samples in this study were prepared by the latter technique. The charged electrodes are heated overnight in an oven at 110°C to remove any absorbed moisture from the arcing mixture.

This mode of sample preparation is particularly desirable in biological analyses where limited sample size is such an important consideration. The success of the method depends on a phenomenon described by Morrison et al. (*9*) and confirmed by this investigation. For biological ash samples, it is possible to use a relatively broad range of sample sizes without markedly affecting the background-corrected emission intensity. By combining total energy burns with a constant internal standard concentration, satisfactory analyses representing micrograms per electrode are accomplished.

Outline of Procedure

Samples and standards, prepared in the manner described, are packed into carbon electrodes and analyzed by a total energy burn in a controlled argon-oxygen atmosphere with dc arc excitation. Spectra are recorded on Eastman S.A. No. 1 film. The lines of interest are densitometered, intensity ratios are calculated, and concentrations are determined as μg/electrode by reference to analytical curves. The analytical lines used are listed in Table 3.

Table 3. Analytical lines employed.

Wavelength, Å			
Ge	Pb	Mn	Pt
2691.34	2833.18	2576.10	3064.79
2709.63	2613.07	2605.69	2997.97
3039.06		2933.06	2929.79
			2659.45

Table 4. Comparison of detection limits.

Element	Detection limit, μg/electrode			
	Koirtyohann and Feldman (*10*)	Morrison et al. (*9*)	Stewart Labs. tissue method	Stewart Labs. custom method
Mn 2576.1	0.008	0.003	0.006	0.003
Pb 2833.1	0.012	0.02	0.02	0.015
Pt 3064.7	—	0.1	0.05	0.010

Table 5. Confirming analyses for platinum, lead, and manganese by atomic absorption spectroscopy.

Element	Sample	Concentration, μg/g wet tissue	
		Emission	Atomic absorption
Pt	14-B-G-LV-b	38.4	40.
	14-B-P-LV-b	30.0	32.
	14-B-G-KL-b	45.7	40.
	14-B-P-KL-b	27.7	<30.
Pb	16- -G-KL-b	14.6	16.5
	16- -P-KL-b	7.48	9.00
	16- -R-KL-b	9.43	10.4
	16- -W-KL-b	21.8	24.4
Mn	16- -R-H-b, 16- -W-H-b	0.97[a]	0.98, 0.72
	16- -G-KL-b	1.22	1.54
	16- -P-KL-b	0.75	0.87
	16- -R-KL-b	1.35	1.38
	16- -W-KL-b	2.18	2.29
	12-3-G-LV-b	4.23	3.40
	12-2-P-LV-b	3.23	3.06
	12-1-R-LV-b	2.42	2.50
	12-4-W-LV-b	1.87	2.09
	17-2-G-LV-b, 17-3-P-LV-b	3.75[a]	3.23[a]
	17-1-R-LV-b, 17-4-W-LV-b	2.57[a]	2.16[a]
	9-1-R-BL-b	22.1	19.9

[a] Composite samples.

Analysis Capabilities

The analytical method, as developed, achieved the desired goal of improving platinum sensitivity by a factor of five over existing methods. A means was provided for evaluating initial exposure experiments on test animals.

Limits of Detection

The limiting sensitivity of the method represents the absolute weight of element

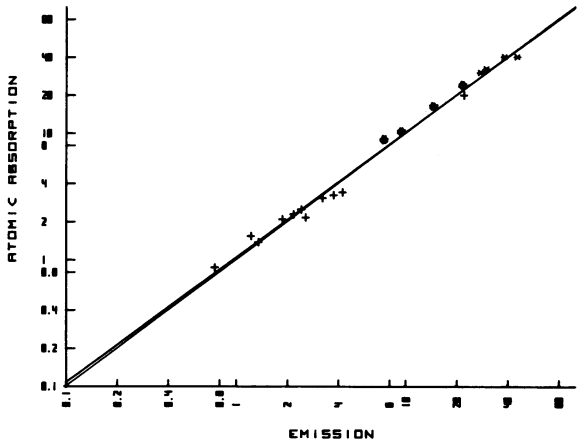

FIGURE 2. Comparison of atomic absorption and emission spectroscopy: (*) platinum; (#) lead; (+) manganese.

necessary to produce an average signal to background ratio of 1:3. Detection limits for this method are compared with those of three other spectrographic techniques employed in the analysis of biological tissues (Table 4).

Precision

The precision of the method, based on multiple analyses of a synthetic tissue sample, is ±10% at the 1 mg level.

Accuracy of Method

Comparative data for manganese, lead, and platinum obtained from the spectrochemical method and atomic absorption are given in Table 5. A plot (Fig. 2) comparing the two sets of independent analyses indicate excellent agreement for the elements studied. A least-squares fit of the data in Figure 2 produced a curve with a slope of 44.4°.

Results and Discussion

The data obtained from this study show that platinum and lead are selectively concentrated by certain tissues while manganese concentrations closely resemble the control distribution (Tables 6–9), The platinum distribution confirms the conclusions drawn from the radioisotope studies reported by Durbin (*7*) in 1960; namely, kidney, liver, and spleen are the chief deposition sites of the platinum metals and were the only tissues that consistently showed higher concentrations than the blood.

Table 6. Analytical data: platinum intraperitoneal study. [a]

SLI code	Sample type	Concentration, μg/g wet tissue		
		Pt	Mn	Pb
FF 001	Liver	38.4	2.01	0.35
FF 002	Liver	30.1	3.24	3.01
FF 003[b]	Blood	0.96	0.45	0.13
FF 004[b]	Blood			
FF 005	Testes	0.91	0.23	0.091
FF 006	Testes	1.53	0.31	0.36
FF 007[b]	Heart	2.96	0.83	0.71
FF 008[b]	Heart			
FF 009	Brain	0.074	0.74	0.21
FF 010	Brain	1.13	0.57	0.11
FF 011	Kidney	45.7	1.96	0.078
FF 012	Kidney	27.7	1.04	0.69
FF 013	Spleen	19.8	0.85	0.40
FF 014	Spleen	11.7	0.59	0.18

[a] Project code: EPA 03048; experiment: $Pt(SO_4)_2 \cdot 4H_2O$; duration: LD_{50}; route of administration: intraperitoneal.

[b] Composite samples.

Table 7. Analytical data: platinum diet study. [a]

SLI code	Sample type	Concentration, μg/g wet tissue		
		Pt	Mn	Pb
FF 015	Liver	3.47	2.70	0.054
FF 016	Liver	1.22	2.45	0.061
FF 017[b]	Blood	3.31	1.32	0.26
FF 018[b]	Blood			
FF 019	Testes	0.58	0.40	0.29
FF 020	Testes	0.39	1.69	0.84
FF 021[b]	Heart	0.76	1.55	0.32
FF 022[b]	Heart			
FF 023	Brain	0.14	1.85	0.14
FF 024	Brain	0.070	3.00	0.20
FF 025	Kidney	19.0	0.76	0.032
FF 026	Kidney	13.4	1.34	0.067
FF 027	Spleen	2.34	0.28	0.37
FF 028	Spleen	4.17	3.13	2.08

[a] Project code: EPA 03048; experiment: $Pt(SO_4)_2 \cdot 4H_2O$ diet; duration LD_{50}; route of administration: oral.

[b] Composite samples.

Table 8. Analytical data: manganese diet study. [a]

SLI code	Sample type	Concentration, μg/g wet tissue		
		Pt	Mn	Pb
FF 043	Liver	0.17	1.34	0.25
FF 044	Liver	0.097	2.53	0.045
FF 045	Blood	0.051	0.25	0.041
FF 046	Blood	0.056	0.64	0.12
FF 047	Testes	0.029	0.40	0.017
FF 048	Testes	0.036	0.53	0.030
FF 049[b]	Heart	0.043	0.71	0.57
FF 050[b]	Heart			
FF 051[b]	Brain	<0.010	0.034	<0.014
FF 052[b]	Brain			
FF 053	Kidney	0.078	1.01	0.78
FF 054	Kidney	0.031	1.53	0.12
FF 055	Spleen	0.51	1.10	<0.015
FF 056	Spleen	0.30	1.48	<0.030

[a] Project code: EPA 03048; experiment: $MnCl_2 \cdot 4H_2O$ diet; duration LD_{50}; route of administration: oral.

[b] Composite samples.

Table 9. Analytical data: lead diet study. [a]

SLI code	Sample type	Concentration, μg/g wet tissue		
		Pt	Mn	Pb
FF 057	Liver	0.58	2.47	1.65
FF 058	Liver	0.25	1.48	0.99
FF 059	Liver	0.30	1.22	0.76
FF 060	Liver	0.34	1.35	1.35
FF 061[b]	Blood	<0.012	0.079	0.079
FF 062[b]	Blood			
FF 063	Blood	0.034	0.84	0.50
FF 064	Blood	<0.0090	0.36	0.12
FF 065	Testes	<0.0083	0.17	0.022
FF 066	Testes	<0.0079	0.11	0.026
FF 067	Testes	<0.0094	0.31	0.031
FF 068	Testes	0.019	0.32	0.097
FF 069[b]	Heart	<0.038	1.26	0.50
FF 070[b]	Heart			
FF 071[b]	Heart	<0.036	0.97	0.48
FF 072[b]	Heart			
FF 073	Kidney	0.24	1.22	14.6
FF 074	Kidney	0.075	0.75	7.48
FF 075	Kidney	0.34	1.35	9.43
FF 076	Kidney	0.94	2.18	21.8
FF 077[b]	Spleen	<0.026	1.23	0.12
FF 078[b]	Spleen			
FF 079[b]	Spleen	<0.034	1.57	0.067
FF 080[b]	Spleen			

[a] Project code: EPA 03048; experiment: $PbCl_2$ diet; duration 90–91 days; route of administration: oral.

[b] Composite samples.

Table 10. Analytical data: rat feed and deionized water.

SLI code	Sample type	Concentration, μg/g (unless otherwise noted) as received		
		Pt	Mn	Pb
FF 248	Feed	0.24	66.6	0.96
FF 249	Feed	<0.02	49.4	0.45
FF 250	Feed	0.14	52.4	1.40
FF 251	Feed	0.13	78.9	0.93
FF 252	Feed	0.05	53.2	0.75
FF 253	Feed	0.10	58.5	1.00
FF 254	Feed	0.08	39.4	1.00
FF 255	Feed	<0.02	74.0	1.28
	Average	0.09	59.1	0.97
FF 417	Deionized water	<0.2[a]	3.[a]	2.[a]

[a] Concentration μg/l (ppb).

Table 11. Analytical data: autopsy tissues. [a]

Sample	Platinum concentration, μg/g wet tissue	
	Liver	Kidney
03002601	1.0	<0.015
02006501	0.07	<0.015
02007001	1.5	0.09
02005801	<0.015	<0.015
02007101	<0.015	<0.015
02006201	<0.015	<0.015
02005001	<0.015	<0.015
02000601	<0.015	<0.015
02000101	<0.015	<0.015
04015501	<0.015	<0.015

[a] Project code: EPA 03172; experiment: 20 autopsy tissues.

The method has also been applied to the analysis of the food and deionized water used in the animal exposure experiments (Table 10) and to the analysis of selected human autopsy tissues (Table 11). The presence of detectable amounts of platinum in the rat food is also suggested by the positive platinum values for kidney and liver tissues from the 90-day lead and manganese feeding studies.

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